Practical Human Growth Hormone

Preparation and Clinical Use*

W. P. VANDERLAAN, M.D., U. J. LEWIS, Ph.D.,†
A. E. JONES, M.D.,† and J. N. FISHER, M.D.,† La Jolla

• **Human growth hormone was prepared from acetone-dried pituitary powder by hot glacial acetic acid extraction and subsequent precipitation by sodium chloride and cold acetone. The yield was 13 per cent and the preparation was called practical growth hormone in recognition of its complement of corticotropin.**

Treatment of two dwarfs with practical growth hormone in aqueous solution, 1 or 2 mg intramuscularly on alternate days, accelerated the growth rate and there were no physical signs or laboratory indications of adrenal stimulation or other adverse effects. The preparation is recommended for its safety, simplicity and relatively good yield.

THE COMPARATIVE biochemistry of growth hormone has come to importance in recent years through the work of Wilhelmi,[23] Beck et al,[2] Knobil and Greep,[7] Raben[13,15] and Li.[8] Raben[14] recently presented his experiences in the treatment of pituitary dwarfism and of other problems involving failure to grow. We report here a method of preparation of human growth hormone adapted from the procedure described by Raden.[15] It has advantages both in greater simplicity and in greater yield and retains the security against contamination provided by hot glacial acetic acid extraction. Safety being an important consideration, some clinical investigators are fearful of the use of otherwise excellent extraction methods. For example, the methods of Wallace and Ferguson,[21] Wilhelmi[22] and Reisfeld et al[17] have very high yields. In addition, we present our method of treatment of a pituitary dwarf over a period of 51 months and a brief experience in the treatment of a girl in whom the cause of dwarfism remains obscure.

METHODS

Preparation of Human Growth Hormone

Human pituitary glands were stored in acetone until 20 grams were accumulated. They were then homogenized with 60 ml of glacial acetic acid and washed into a beaker with the addition of 200 ml of acid. With continuous stirring the mixture was heated to 70° C, and then allowed to cool to room temperature. It was centrifuged for 15 minutes at 20,000 × *g*, and the precipitate was discarded. The supernatant, having a volume of 226 ml, was cooled to 5° C, and 1.13 ml of 5 M NaCl was added, followed by 56.5 ml of chilled acetone. After filtering at 5° C, the precipitate was discarded and the protein in the supernatant was precipitated with 565 ml of chilled acetone. After centrifugation at 20,000 × *g* the supernatant was discarded, and the precipitate was washed with 50 ml of chilled acetone and the washings discarded. The precipitate was dissolved in 40 ml of 0.1 M ammonium formate buffer pH 5.8. After lyophilization the yield in protein was 13 per cent of the whole dried glands. The dried product usually contained approximately 75 per cent protein, the remainder being buffer salts. The concentration of protein was determined by the method of Sutherland and co-workers,[20] using serum albumin as the standard.

Sterilized glassware was used only after the final washing of the precipitate with acetone. The ammonium formate buffer was prepared with sterile water, and sterilized glassware was used for the lyophilization. The dried material was transferred aseptically to a sterilized, screw-cap vial for storage.

To prepare a sample for intramuscular injection, 30 mg (protein) was weighed into a sterile vial (10 ml capacity) and was suspended in 2 ml of sterile water (water for parenterals, U.S.P., containing 0.9 per cent benzyl alcohol.) To effect solution of the sample, 0.5 ml of 0.1 N HCl (0.25 ml of 12 N HCl added to 30 ml of sterile water) was added from a sterile syringe, and the mixture was drawn up and down in the syringe until the solids dissolved. The solution was then transferred to a bottle having a rubber injection cap and containing 15 ml of sterile water (with preservative).

This preparation, which we have called "practical growth hormone" in recognition of its complement

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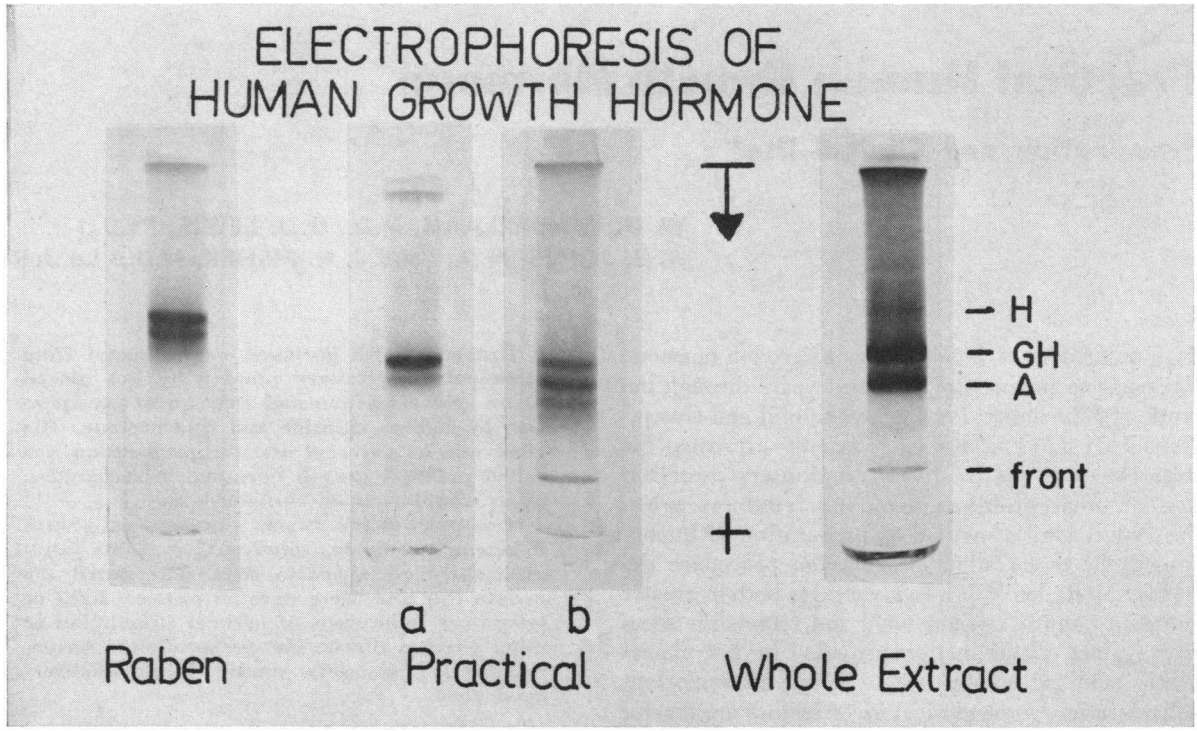

Figure 1.—The results of four studies of human growth hormone by disc electrophoresis carried out at pH 9.0 are shown. The Raben material is shown as having three components, the practical material shows greater homogeneity (a) but after incubation for 48 hours at pH 8.0 (b) shows some degradation, resembling the Raben material. The whole extract of human pituitary gland shows growth hormone in a single heavy band (GH). The band H is hemoglobin and A is albumin. The arrow indicates the direction of motion, and the word *front* indicates the forward moving boundary. The whole extract was made with 0.025 M Na_2CO_3-HCl, pH 10.

of corticotropin, was compared with human growth hormone (Raben) by use of the procedure of disc electrophoresis of Ornstein and Davis.[11] The modification of Reisfeld and coworkers[17] was used for electrophoretic analysis at an acidic pH.

Practical growth hormone was assayed for growth activity by the technique of Greenspan and coworkers,[5] for thyrotropin by McKenzie's method,[10] for corticotropin by that of Sayers and coworkers,[18] and for luteinizing hormone according to Parlow.[12]

Free fatty acids were determined by the method of Dole,[3] 17 hydroxycorticosteroids by the method of Silber,[19] 17 ketosteroids by the method of Drekter,[4] protein-bound iodine by the method of Zak and coworkers,[24] and alkaline phosphatase by a modification of the method by Bodansky.[6]

RESULTS

Analytical

Figure 1 compares the disc electrophoretic pattern of human growth hormone (Raben) with that of the practical growth hormone before and after incubation at pH 8. The electrophoresis was carried out at pH 9. Greater homogeneity was observed in the practical growth hormone than in the Raben material and this appears to be, therefore, due to the avoidance of an alkaline step, in accordance with the observations of Barrett and co-workers[1] and Lewis,[8] who showed that growth hormone degrades under alkaline conditions.

The electrophoretic pattern of the whole pituitary extract showed only two prominent components. The major band (GH in Figure 1) behaved electrophoretically like growth hormone. The second principal component (A) moved slightly ahead of the growth hormone and migrated with the same mobility as albumin. There was smearing of reddish-brown material in the upper portion of the gel, probably due to hemoglobin-haptoglobin complexes. There was one component (H) that migrated as human hemoglobin, however. The high yield of growth hormone from human pituitary glands[21,23,17] is explained by the electrophoretic pattern since apparently the major extractable protein of the gland is growth hormone.

The results of electrophoresis by the adaptation of Reisfeld and coworkers[17] of Ornstein and Davis' method[11] to an acidic pH are shown in Figure 2. In this method the Raben material ran as a single component. The separation of corticotropin and other basic proteins from growth hormone by treatment with oxycellulose is highly suggested by the electrophoretic study of the practical material. The

TABLE 1.—*Data on Treatment and Results, Case 1*

Date	Age Yr.	Age Mo.	Ht. Inches	Wt. Lb.	Treatment: Raben Growth Hormone	Treatment: Practical Growth Hormone	Treatment: Thyroid USP mg daily	Treatment: Cortisone mg daily	Treatment: Testosterone cyclopentyl-propionate mg 2 weeks	Serum Alkaline Phosphatase Bodansky Units	Wrist Bone Age Yr.	Wrist Bone Age Mo.
March '43		Birth	21	6+	.....	.....	.....	.....	.....	.....	.....	
Feb. '56	12	11	49	12	.....	.....	.....	.....	.....	.....	7	6
Sept. '58	15	6	52	...	.....	.....	.....	.....	.....	3.9	10	0
Nov. '58	15	8	52	92	2 mg/48 hr	.....	.....	.....	.....	.....	.....	
Dec. '58	15	9	52½	93	2 mg/48 hr	.....	120	10	.....	6.4, 5.3	.....	
March '59	16	..	53	90	2 mg/48 hr	.....	120	10	.....	.....	11	9
Sept. '59	16	6	54½	95	2 mg/48 hr	.....	120	10	.....	10, 8.6	.....	
March '60	17	..	55½	105	2 mg/48 hr	.....	120	10	.....	.....	.....	
June '60	17	3	56	105	2 mg/48 hr	.....	120	10	.....	9.0	12	6
Nov. '60	17	8	56¾	109	4 mg/48 hr	.....	120	10	100	6.1	.....	
March '61	18	..	57½	111	4 mg/48 hr	.....	120	10	100	.....	.....	
June '61	18	3	59½	.....	2 mg	.....	120	10	100	.....	.....	
Oct. '61	18	7	60	121	.....	2 mg	120	10	200	.....	14	0
March '62	19	..	60¾	134	.....	2 mg	120	10	200	9.2	.....	
Oct. '62	19	7	62	137	.....	5 mg	120	10	200	7.0	16	6
March '63	20	..	63	137	.....	5 mg	120	10	200	4.9	.....	

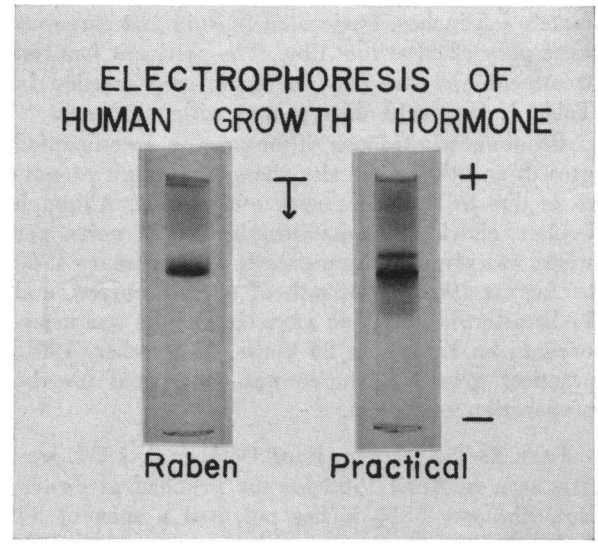

Figure 2.—Electrophoresis at an acidic pH shows human growth hormone prepared by Raben's method as a single component. Failure to remove corticotropin in the practical material is probably reflected in the additional bands seen. The direction of flow between poles is indicated by the arrow.

three components observed in Raben's method during electrophoresis at pH 9 were not received when analyzed at pH 4.3.

The practical material was found to be devoid of thyrotropic activity when 20μg was injected into each mouse. Corticotropin activity was 300 units per milligram and luteinizing hormone was not demonstrable.

Clinical

CASE 1. September, 1958, a boy aged 15 years and 6 months was referred to this clinic for evaluation of endocrine status and the problem of headache. He was nearly 52 inches tall, symmetrical, sexually infantile and clinically a pituitary dwarf. This opinion was reinforced by the moderate retardation of bone age (ten years), alkaline phosphatase of 3.9 Bodansky units, I^{131} uptake of 8 per cent in 24 hours, and 17 ketosteroid excretion of 2.3 mg in 24 hours. The patient was discharged until human growth hormone was available. Pituitary glands were collected at autopsy, and the initial preparation of human growth hormone was made by Raben.*

For six weeks human growth hormone (Raben) was administered intramuscularly, 2 mg alternate days without the addition of desiccated thyroid or of cortisone. At the end of six weeks serum protein-bound iodine had fallen from 5.4 to 3.1μg per 100 ml. Alkaline phosphatase activity increased, as recorded in Table 1, and height increased approxi-

*We are indebted for helpful advice and for the initial supply of human growth hormone to Dr. Maurice S. Raben, Boston, Massachusetts.

mately 0.5 inches. Desiccated thyroid and cortisone were prescribed at that time. The pertinent facts on treatment and the response to it are recorded in Table 1. Headache disappeared with treatment.

Pronounced evidence of maturation accompanied growth and the penile size changed to adult proportions due to treatment with testosterone. Although human chorionic gonadotropin, 1500 units per week, was given intramuscularly from January 1960 to August 1961, no growth of testes occurred, and 17 ketosteroid excretion after six months was measured to be 1.8 mg in 24 hours. In October, 1961, practical growth hormone was substituted for the preparation of Raben.

CASE 2. The patient, born October 8, 1954, was first seen in April 1962 for the problem of dwarfism. She was 35½ inches tall, had a span of 34 inches between fingertips with the arms outspread, and weighed 22 pounds. Her parents had moved from Michigan to this area in the misunderstanding that human growth hormone was exclusively available here. She had been extensively investigated in Boston in March and April, 1958, for failure to grow. Examination at that time had disclosed the following: height 29 inches, weight 15 pounds, normal proportions, and, except for mild eczema, no other abnormalities on general examination. Bone age was at the one-year level. Despite extensive examination the basis of failure to grow was not determined.

Physical examination showed acne rosacea and extremely small stature in a bright and alert youngster.

Studies of blood (hemoglobin 11.3 grams per 100 ml), of urine (specific gravity 1.016), alkaline phosphatase activity (5.5 Bodansky units), 17 ketosteroid excretion (0.88 mg in 24 hours) and 17 hydroxycorticosteroid excretion (0.84 mg in 24 hours), protein-bound iodine (6.9 μg per 100 ml) and I^{131} uptake of 20 per cent in 24 hours, failed to clarify the diagnosis. X-ray studies of the skull and long bones showed a bone age of 2 years 6 months, but no other abnormality. Growth hormone assay and sulfation factors in serum were 83 μg per ml and 32, respectively.† Chromosome studies on an aspirate of bone marrow indicated a normal pattern, and buccal and leukocyte studies were chromatin-positive.‡ After the patient had fasted overnight, the free fatty acid concentration in the plasma was 1275 microequivalents per liter, rising to 2150 four hours after the intramuscular injection of 2 mg of practical growth hormone.

In the absence of a diagnosis, empirical treatment with practical growth hormone was begun in dosage of 1.0 mg every 48 hours intramuscularly. In the first month there was no change, and in the next four months there was one full inch of growth and a weight gain to 25¼ pounds.

DISCUSSION

Much emphasis recently has been put on the desirability of purity in the preparation of growth hormone, and the press has carried accounts indicating human use should be deferred to make available all material for structural studies in the hope of eventual synthesis. We disagree. It is our position that proper utilization of available sources of human pituitary glands will allow clinical study to proceed, defining the place of the preparation in therapeutics and alleviating the great and largely avoidable suffering entailed in hypopituitary dwarfism, without seriously encroaching upon chemical studies.

We have described a simple method for the preparation of human growth hormone and the results of its use in two patients. Optimal dosage of the material thus prepared has not been established but growth hormone assay by the tibial technique and our clinical experience indicate it to be comparable in potency to the Raben preparation.

The avoidance of an alkaline step in the procedure probably is of advantage, judging by the higher yields. Failure to remove corticotropin poses a disadvantage for careful metabolic studies, but in clinical observation no evidence has been seen of adrenal stimulation. The intramuscular injection of an aqueous preparation of corticotropin every 48 hours would not be expected to have readily detectable effects.

Some comment may be in order on the patient in Case 1. He has been treated for 51 months with a net gain of 11 inches in height. At the beginning of treatment the goal mutually agreed upon was five feet in height. When he was about 4 feet 9 inches tall, the addition of testosterone was made for several reasons. Our supply of growth hormone was insufficient to maintain the dosage of 4 mg per 48 hours, he was within 3 inches of the goal of treatment for height, and he was at the upper limit of age for the normal appearance of puberal changes. We have also held to an arbitrary decision to have achieved the full growth before the age of 21 on the basis that rapid growth to a reasonable height by this age appears more desirable than the achievement of somewhat greater height at a considerably later date. The greatest rate of growth was observed while he was treated with the larger dose of growth hormone, 4 mg in 48 hours, plus testosterone cyclopentyl-propionate, 100 mg in two weeks.

476 Prospect Street, La Jolla, California 92038 (VanderLaan).

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